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**EVALUATION OF DIGITAL IMAGING STAY-GREEN AS A METHOD OF INDIRECT SELECTION
FOR GRAIN YIELD IN MAIZE (*Zea mays* L.)**

by

Juan Emigdio Beltran

A creative component submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Breeding

Program of Study Committee:

Dr. Anthony Assibi Mahama, Major Professor

Dr. Thomas Lubberstedt

Iowa State University

Ames, Iowa

2020

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NOMENCLATURE

UAD	unmanned aerial device
YOR	year of release
RGB	red green blue computerized values

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ABSTRACT

Stay-green has been used in the past as a secondary trait for indirect selection in grain yield for maize. Stay-green is usually successfully used in stressed environments, low nitrogen and drought, but tends to have poor heritability in the absence of stress compared to grain yield, in contrast to grain yield which tends to drop under stressed environments. This leads to stay-green being overshadowed by other popular secondary traits such as anthesis silking interval. Some of the variability in stay-green is produced by subjectivity. With the use of unmanned aerial devices or drones becoming more popular, imaging was taken for a set of ERA Pioneer hybrids grown under well-watered and drought stress treatments to eliminate statistical noise and improve heritability and correlations. Images for these plots produced RGB values which were then converted to a green leaf index to substitute for conventional stay-green.

Genetic correlation and heritability values were computed for full water and drought stress conditions, with drought stress as the control. Heritability was high for both grain yield and stay-green. Indirect selection efficiency was found to still be more efficient under a drought stress condition. However, UAD stay-green was able to provide significance values for the full water condition, something rarely found in previous research with conventional stay-green for non-stressed environments.

EVALUATION OF DIGITAL IMAGING STAY-GREEN AS A METHOD OF INDIRECT SELECTION FOR GRAIN YIELD IN MAIZE

CHAPTER 1

INTRODUCTION

Costs are no longer the same as they were 20 years ago to run a research farm or any farm. As wages for research workers have increased, so has readily available technology advanced. The goal for all plant breeders is to increase genetic gain in their crop. A breeder can work with a broad germplasm with highly heritable traits and increase the number of testing locations and/or replications to decrease phenotypic variance and increase genotypic variance. However, any breeding program of any size is unsuccessful if it does not have the finances to support the high costs associated with planting to analyzing data and making decisions.

The most important trait in maize is grain yield. However, grain yield tends to have poor heritability. Under drought stressed conditions, heritability can drop from 0.60 to 0.32 (Ziyomo & Bernardo, 2012). As such, any person that works in agriculture can attest to the fact that no two seasons are ever the same, and maize, no matter how well taken care of, will always be vulnerable to high or low temperatures, high winds, other abiotic and biotic factors. Under optimal conditions, heritability of grain yield can be as high as 0.92 and drop to 0.55 with random abiotic stress (Weber et al., 2012). For cases such as this, indirect selection methods have been developed to make smart decisions regardless of stress conditions and low heritability of grain yield (Badu-Apraku et al., 2012; Ziyomo and Bernardo, 2012). Indirect selection involves selecting a secondary trait, e.g. plant height, stay-green, anthesis silking interval, to improve performance in a primary trait, such as grain yield. Badu-Apraku et. al. (2012) were able to find efficiency improving grain yield by selecting stay-green, plant height, and ear height under low Nitrogen environments. Similarly, Ziyomo and Bernardo (2012) were able to improve grain yield by selecting leaf senescence, anthesis silking interval, and plant height under drought conditions. The indirect method of selection is only efficient if there is a strong genetic correlation between the primary trait and the secondary trait, and if the heritability of the secondary trait is higher than the primary trait (Bernardo, 2010). This concept makes indirect

selection an important method for a plant breeder to use in cases where they cannot select directly for the primary trait.

A technology that has become increasingly useful during the last decade is the use of unmanned aerial devices (UAD) for phenotypic evaluation in the field. A camera can be mounted on to a drone and flown to take pictures of plots. Multiple images are later stitched together in a geographic information system (GIS) mapping software (Stehr, 2015). Depending on software or the sophistication of the camera lens, it can measure numerous traits, e.g. plant health, height, disease pressure, and plant number (Stehr, 2015; Sykes et al., 2017). A UAD can measure plots within an hour, whereas with manual labor, it could take days to complete. In one study, time to complete reading on an experimental location was reduced from 2 hours to 15 minutes when using aerial imagery (Inostroza et al., 2016). It is easy to imagine how much time and labor can add up to, when considering several experimental locations. This tool has become very critical as the wages for labor have steadily increased over the years. Moreso, UAD reduces sampling error from differences due to subjectivity between personnel or even from the same personnel throughout different times of the day. Sykes et al. (2017) found that readings were more precise with smaller standard deviations in using digital imagery compared to visual estimates. This can decrease phenotypic variance due to error and increase the heritability for the given trait.

A secondary trait that is of interest to maize breeders is stay-green. Stay-green is an indicator of absence of stress, retained chlorophyll content, increased duration of photosynthetic productivity, and delayed leaf senescence (Smith, et al., 2004; Woo, Kim, Nam, & Lim, 2013; Prasad & Staggenborg, 2008). Stay-green has been an important trait in sorghum, where it has been used successfully as a secondary trait in indirect selection for grain yield, especially in drought tolerant varieties (Burke, Franks, Burow, & Xin, 2010). It has been used in maize in a variety of different programs, where the genetic correlation is comparable to sorghum; $r = -0.75$ compared to $r = -0.76$ in maize (Burke et al., 2010; Ziyomo & Bernardo, 2012). However, this genetic correlation is strong under stressed conditions, where it can increase to -0.65 in a stressed condition such as low Nitrogen (Badu-Apraku, Akinwale, Franco, & Oyekunle, 2012), and decrease to -0.29 in non stressed condition such as no drought (Ziyomo & Bernardo, 2012). A wide range of heritabilities of stay-green in maize was found depending on type of environment,

usually low under well controlled environments. Stay-green also can have a higher heritability(0.61) than grain yield (0.37) under the same conditions of drought (Ziyomo & Bernardo, 2012) .

Measuring stay-green involves someone walking plots and rating “greenness” of the canopy, usually on a 1-10 point scale. This can be highly subjective depending on, whether it is being done by different people, optical strain on one person, or different lighting conditions throughout the day. However, with stay-green measured by UAD imaging, there might be room for improvement in heritability by reducing residual variance and strengthening the genotypic to phenotypic variance ratio due to its precision. An evaluation of UAD stay-green on Pioneer top selling hybrids from the last half century will provide information useful for testing of indirect selection.

Our objectives in this study were to use UAD stay-green from these hybrids grown in a fully watered condition and under drought stress, to determine if UAD can improve broad sense heritability and genetic correlation with grain yield, and if it can improve the efficiency of indirect selection regardless of environment.

CHAPTER 2

MATERIALS AND METHODS

This study was conducted in Woodland, CA at a Corteva research station during the 2018 season. The study was an ERA type concept, similar to that popularized by Dr. Donald Duvick, where the top performing hybrids of each year are observed together to distinguish obvious changes in primary traits such as yield, or secondary traits such as plant architecture, stay-green, anthesis silking interval, etc. (Debruin et al. 2017; Duvick et al. 2004; Reyes et al., 2015; Smith et al., 2004). The study included 17 Pioneer brand hybrids with year of release (YOR) ranging from 1930 to 2014 (Table 1). Hybrids before 1960, represent double cross hybrids popular at the time, single cross hybrids were used post 1960. There was a gap of hybrids between 1955-1981 due to seed availability (the target number was 20 hybrids, but 3 were replaced by filler seed). The study was tested under two different water management conditions leading up to flowering. These are, full water (plants were given adequate amounts of water), and drought stress (plants were given minimal water). The entries under each condition were replicated twice.

Table 1. Hybrids with Year of Release

Hybrid	YOR
Reid YD	1930
322HYB	1936
330HYB	1939
340HYB	1941
339HYB	1942
352HYB	1946
347HYB	1950
301B	1952
354HYB	1953
329HYB	1954
3377	1982
3394	1991
33P67	1999
34H31	2002
33D49	2007
P1151HR	2010
P1197YHR	2014

The study was flown over by a UAD that took images at 5 weeks before the experiment was harvested. Images were converted to RGB (Red, Blue, Green) Color Model values, based on computer algorithms for colors (El-Sheimy, Lari, and Hassanein, 2018). To make this information practical to humans, the RGB values were converted to a Green Leaf Index, using a formula provided by a public website, *Index DataBase* (Henrich, et al. 2020), which accounts for leaf greenness in an image, and will substitute for stay-green values in this study. *Index Database* provides many indices for greenness, but Green Leaf Index was chosen due to its significant strength found in this statistical model. Grain yield was measured by a New Holland TR series research plot combine set for 0.15 g g⁻¹ moisture.

A randomized complete block design was used for the experiment where replications of hybrids (YOR) were assigned to plots at random within two blocks (Figure 1). Based on the linear model, ANOVA were conducted using R code for both grain yield bushels/acre (ton/ha) and stay-green. Plots were two rows of 4.5 m length with 0.60 m alleys and 0.76 m spacing between rows. Plants were densely spaced at 33 plants in a single row, amounting to nine plants per square meter population density.

	Full Water													Drought Stress																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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Figure 1. Experiment Layout.

The linear model used to establish distinctness for the two conditions is

$$Y_{ijk} = \mu + YOR_i + Block_j + YOR * Block_{ij} + \epsilon_{ijk},$$

where

Y_{ijk} is either stay-green or grain yield (bu/ac)

μ is the grand mean

YOR_i is year of release of the Pioneer brand hybrid, where $i=1,2, \dots, 17$ levels

$Block_j$ is the water management condition, where $j= 1$ or 2 level

ϵ_{ijk} is the residual effect not accounted for in the model

For each condition, the linear model used is

$$Y_{ij} = \mu + YOR_i + \epsilon_{ij}$$

where

Y_{ij} is either stay-green or grain yield (bu/ac)

μ is the grand mean

YOR_i is year of release of the Pioneer brand hybrid, where $i=1,2, \dots, 17$ levels

ϵ_{ij} is the residual effect not accounted for in the model

Variance components were calculated from ANOVA outputs using Method of Moments (Table 2). Broad Sense heritability was calculated for both yield bu/ac (ton.ha) and stay-green by $H = \frac{\sigma_G^2}{\sigma_P^2}$, where σ_G is genotypic variance, and σ_P is phenotypic variance (Bernardo, 2010).

Genetic correlation was calculated by $r_G = \frac{Cov_{G(xy)}}{\sqrt{(V_{G(X)}V_{G(Y)})}}$, where $Cov_{G(xy)}$ is the genetic covariance between yield and stay-green. $V_{G(X)}$ is the genetic variance of stay-green, and $V_{G(Y)}$ is the genetic variance of yield (Bernardo, 2010).

Indirect selection efficiency was calculated by $\frac{R_Y^C}{R_Y} = \frac{|r_G| h_x}{h_y}$, where $|r_G|$ is the absolute value of the genetic correlation between yield and stay-green, H_x is the square root of the heritability of the secondary trait stay-green, and H_y is the square root of the heritability of the primary trait grain yield (Bernardo, 2010).

Table 2. Variance Components

Source of Var	Df	SS	MS	F	Prob F	EMS
Total	rtb-1; 68-1 = 67	SSTot				
YOR, t; 17	t-1; 17-1 = 16	SS _t	SS _t /(t-1)			$\sigma_e^2 + rt\theta_t^2$
Block, b; 2	b-1; 2 - 1 = 1	SS _b	SS _b /(b-1)			$\sigma_e^2 + rt\theta_b^2$
Block x YOR	(t-1)(b-1); 17 - 1 = 16	SS _{tb}	SS _{tb} /[(t-1)(b-1)]			$\sigma_e^2 + r\theta_{tb}^2$
Error, E	tb(r-1); 34	SS _E				σ_e^2

CHAPTER 3

RESULTS

For both full water and drought stress conditions (also referred to as blocks), we observed highly significant differences among YOR for grain yield performance and water conditions (Table 3, Table 4). There were larger phenotypic variances for grain yield under full water condition compared to drought stress condition. Similarly, we observed highly significant differences among YOR for stay-green performance. Water conditions were different, with larger phenotypic variances under drought stress (Tables 5, 6). This was expected due to stay-green's ability as a stress indicator in drought stress or other stressed conditions (Weber, et al., 2012). Heritability for stay-green was calculated from these variance components, and was slightly higher under drought stress than under full water condition. In contrast, heritability was higher for grain yield under a full water condition compared to drought stress conditions (Table 7).

Table 3. ANOVA for grain yield under full water and drought stress (whole experiment)

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	16	269121	16820	250.666	< 2.2e-16	***
Block	1	13286	13285.9	197.9968	1.73E-12	***
YOR x Block	15	5165	344.3	5.1317	0.00008318	***
Residuals	29	1946	67.1			

*** = Significant at 0.001

Table 4. ANOVA for grain yield under full water versus drought stress

Full water						
Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	16	162448	10153	268.99	2.227E-16	***
Residuals	16	604	37.7			
Drought Stress						
Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	15	116588	7772.5	75.291	3.863E-10	***
Residuals	13	1342	103.2			

*** = Significant at 0.001

Table 5. ANOVA for stay-green under full water and drought stress (whole experiment)

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	16	0.030991	0.001937	33.5446	3.46E-16	***
Block	1	0.001273	0.001273	22.0422	4.26E-05	***
YOR x Block	16	0.00131	8.19E-05	1.4176	0.1915	
Residuals	34	0.001963	5.77E-05			

*** = Significant at 0.001

Table 6. ANOVA for stay-green under full water versus drought stress

Full water						
Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	16	0.0113	0.00071	24.056	1.39E-08	***
Residuals	17	0.0005	2.9E-05			
Drought Stress						
Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
YOR	16	0.021	0.00131	15.24	4.716E-07	***
Residuals	17	0.00146	8.6E-05			

*** = Significant at 0.001

Table 7. Variance Components and Broad Sense Heritability

	grain yield			stay-green		
	Genotypic	Phenotypic	H ²	Genotypic	Phenotypic	H ²
Whole experiment	4118.925	4746.189	0.867838	0.00046377	0.00053	0.87438
Full water	5057.65	5095.362	0.992599	0.00034	0.000358	0.946576
Drought stress	3834.5	4449.283	0.861824	0.00066	0.000681	0.96404

Genetic correlation between grain yield and stay-green was calculated for each of the conditions, with genetic correlations being closer under drought stress (Table 8). These results differ from the aforementioned heritability values of grain yield, where it was higher at full water supply. However, genetic covariance between grain yield and stay-green was higher under drought stress conditions leading to that closer correlation.

Table 8. Covariance and Genetic Correlation of stay-green and grain yield

	Covariance	Genetic Correlation
Full water	1.049831	0.802395
Drought stress	1.474672	0.929615

Indirect selection efficiency was calculated for each of the two conditions. Due to a closer genetic correlation, lower heritability for grain yield, higher heritability for the secondary trait stay-green, indirect selection was more efficient under drought stress compared to full water, 0.98 and 0.78, respectively (Table 9).

Table 9. Indirect Selection Efficiency of stay-green for grain yield

	Indirect Selection Efficiency
Full water	0.783572
Drought stress	0.983199

CHAPTER 4

DISCUSSION

There were high heritability values for grain yield, 0.99 and 0.86 under full water supply and drought stress conditions, respectively. These values are high compared to values in previous studies for similar conditions, 0.60 and 0.37 (Ziyomo & Bernardo, 2012), 0.92 and 0.44 (Weber, et al., 2012). One possible explanation for heritability being so high is the distinct genotypes being used across 1930-2010 caused a higher genetic variance based on advancements of hybrids in the last eighty years. In the study by Ziyomo and Bernardo (2012), the hybrids tested were F_1 crosses produced by using F_7 inbreds from a B73 and Mo17 population and the same tester. Conversely, in the nine year study of Weber et al. (2012), there were 448 different hybrids tested, allowing for more variability across hybrids, and heritability similar to the full water heritability in this study. There were high heritability values for UAD stay-green produced in this study, 0.95 and 0.96 for full water and drought stress conditions, respectively. These values are extremely high, compared to anything that has been reported when using conventional stay-green readings. In four generations of selection derived from the same F_1 cross, heritability of stay green was very low, as it ranged from 0 to 0.29 (Badu-Apraku, Talabi, Obeng-Bio, & Asiedu, 2018), and was 0.59 for twenty two hybrids derived from a common random mating population (Musvos, Setimela, & Wali C., 2018). In the study by Ziyomo and Bernardo (2012), the F_1 hybrids produced with the same tester had a heritability for stay-green of 0.61 under drought conditions. Heritability was not calculated for stay-green in their full water environment due to not finding significant differences in their linear model for stay-green performance among their hybrids. In this study in Woodland, UAD stay-green was still able to capture significant differences under full water condition. Ideally, grain yield heritability would need to be lower than stay-green heritability in both water conditions for optimal indirect selection. Subsetting hybrids to before 1953, and after 1982, stay-green and grain yield values become less distinct (Figure 2, Figure 3). Perhaps by running this study on current year hybrids in the last 10 years, we could expect genetic variance for grain yield to be smaller but could also possibly weaken the genetic variance in stay-green. The use of UAD did strengthen heritability values of stay-green to use as a secondary trait for indirect selection, regardless of water conditions and distinct genotypes. $g\ m^{-2}$

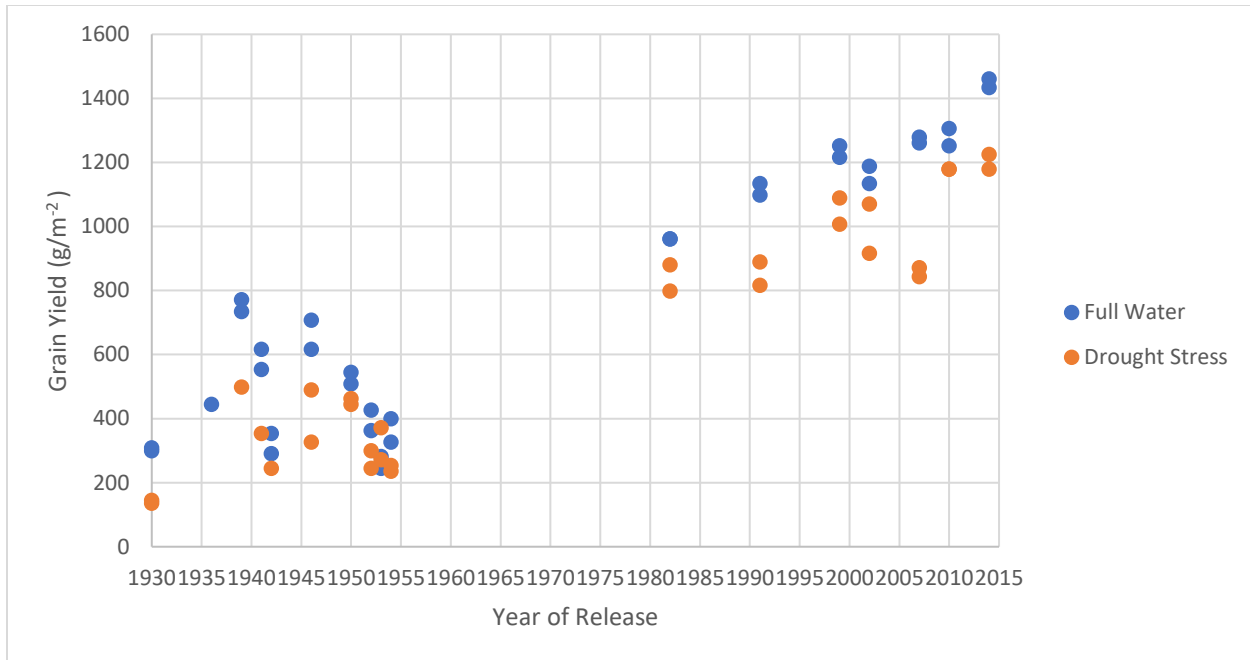


Figure 2. Correlation between grain yield and hybrid Year of Release

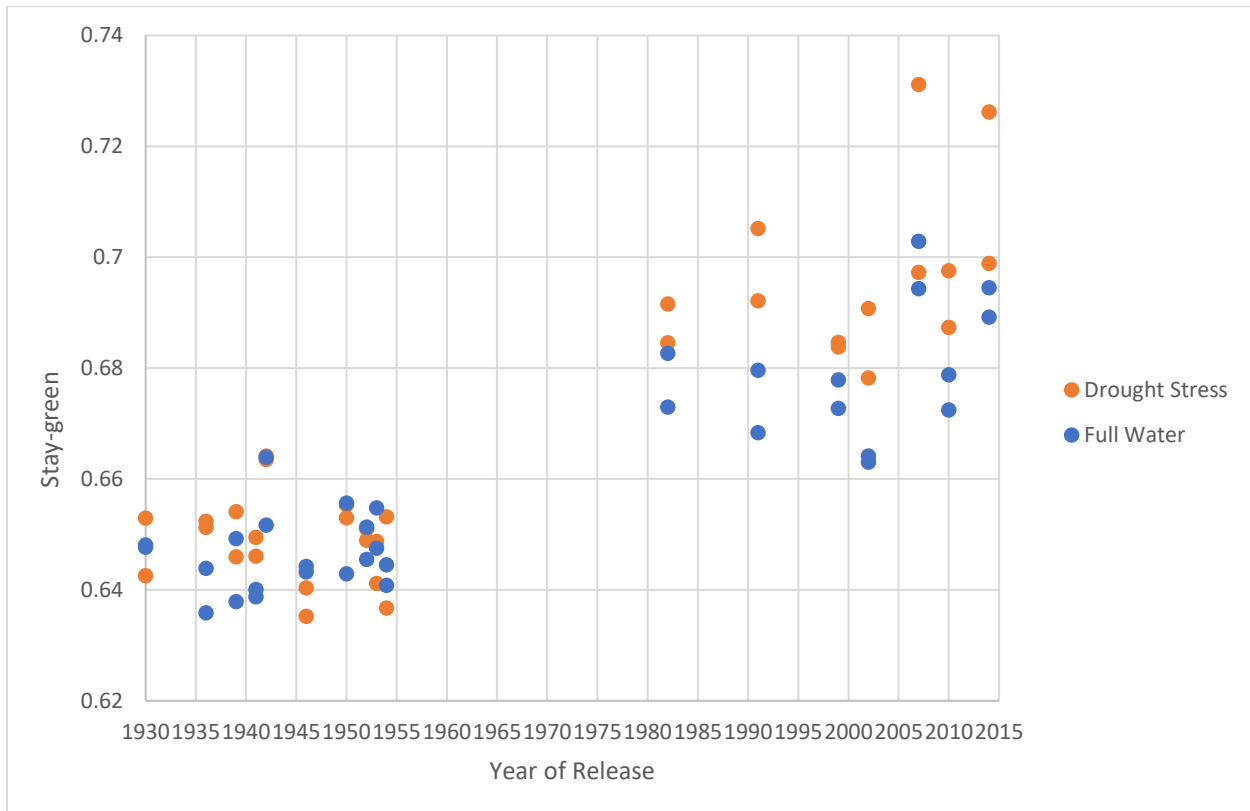


Figure 3. Correlation between stay-green and hybrid Year of Release

The genetic correlation in this study between yield and stay-green were 0.8024 under full water conditions and 0.9296 under drought stress (Figure 4). These were major improvements compared to the genetic correlation values found in the study by Ziyomo & Bernardo(2012), where values were 0.29 and 0.76 for full water and drought stress, respectively. This could be due to either a stronger covariance between grain yield and stay-green values, which is accounted for by less environmental variance produced by UAD provided stay-green, or less genotypic variance of either grain yield or stay-green.

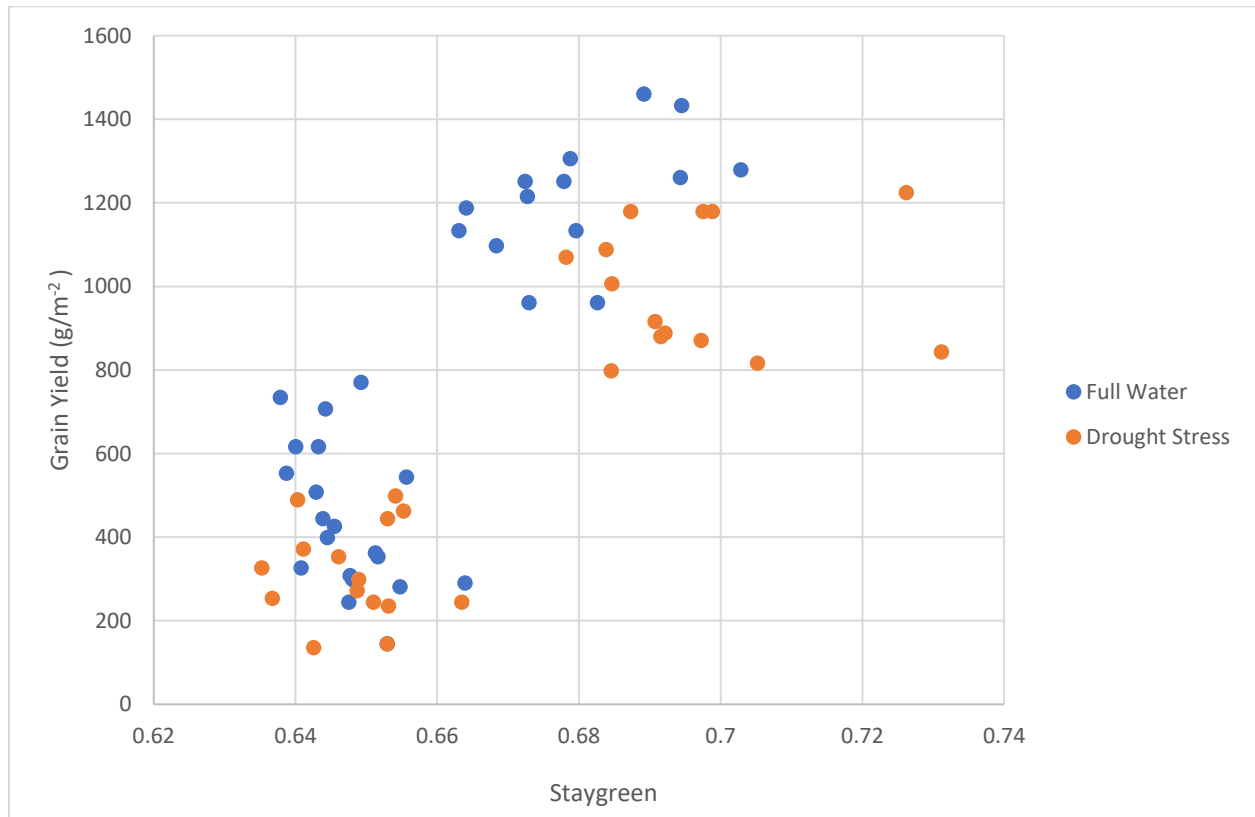


Figure 4. Correlation between grain yield and stay-green

Although, in this study we were not able to show that UAD provided stay-green led to improved indirect selection efficiency under full water condition compared to drought stress condition, UAD stay-green still provides useful data. When employing lsmeans for both grain yield and stay-green, the five best performing hybrids were selected (for 30% selection intensity). Full water use of stay-green was able to choose 80% of the hybrids selected under full water based on grain yield alone, although rankings were not in the same order (Table 10). Conversely, drought stress use of stay-green was only able to choose 60% of top yielding hybrids under drought stress

or full water. Although we had high heritabilities for stay-green in this study, indirect selection efficiency under drought stress of 0.9831 is similar to the 0.98 efficiency reported by Ziyomo & Bernardo (2012). This is due to their lower heritability for grain yield and our higher heritability for grain yield. But as mentioned before, there were no significant differences for stay-green in the fully watered environment for Ziyomo & Bernardo(2012), so we can infer that UAD provided stay-green led to improved indirect selection efficiency compared to conventional stay-green in a full water environment.

Table 10. LSMeans rankings of grain yield (bu/ac) and stay-green under full water and drought stress conditions

YOR	Full water				Drought stress			
	Rank	Yield	Rank	Stay-green	Rank	Yield	Rank	Stay-green
2014	1	253.8	2	0.692	1	210.9	2	0.713
2010	2	224.6	4	0.676	2	207.1	4	0.692
2007	3	222.6	1	0.699	5	151	1	0.714
1999	4	216.8	5	0.675	3	184.4	6	0.684
2002	5	204.2	7	0.664	4	174.6	7	0.684
1991	6	196.4	6	0.674	6	149.5	3	0.699
1982	7	169	3	0.678	7	146.8	5	0.688
1939	8	132.1	13	0.644	8	87.6	11	0.65
1946	9	116.5	14	0.644	10	71.2	17	0.638
1941	10	102.7	17	0.639	11	62.7	13	0.648
1950	11	92.5	10	0.649	9	79.4	9	0.654
1936	12	78	16	0.64	17		10	0.652
1952	13	68.8	11	0.648	13	47.6	12	0.65
1954	14	63.7	15	0.643	15	42.5	16	0.645
1942	15	56.5	8	0.658	14	42.9	8	0.664
1930	16	53.5	12	0.648	16	25.1	14	0.648
1953	17	45.7	9	0.651	12	56.9	15	0.645

CHAPTER 5

SUMMARY AND CONCLUSIONS

In this study, UAD provided stay-green was shown to improve heritability in a full water condition. UAD stay-green as a secondary trait can be used for indirect selection under drought stress conditions. UAD stay-green should still be used as a trait of interest in full water conditions due to the close correlation we found for top yielding ERA hybrids. UAD stay-green when used for indirect selection or some sort of index selection, where multiple traits are being considered, should best be used in populations that are distinct, and is not expected to be highly heritable in closely related populations. As we move to a digital agriculture space, UAD stay-green is/will/could be an important trait for predicting yield performance in maize.

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